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Award Number: W81XWH-08-2-0021

TITLE: Molecular Mechanisms Underlying Individual Differences in Response to Stress in a Previously Validated Animal Model of PTSD

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REPORT DATE: September 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

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# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| ***1. REPORT DATE*** *(DD-MM-YYYY)* | **2. REPORT TYPE** | **3. DATES COVERED** *(From - To)* |
|---|---|---|
| 01-09-2009 | Annual | 01/09/2008 - 31/08/2009 |

**4. TITLE AND SUBTITLE**

Molecular Mechanisms Underlying Individual Differences in Response to Stress in a Previously Validated Animal Model of PTSD

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-08-2-0021

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Bronx Veterans Medical Research Foundation
Bronx, NY 10468-3904

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research And Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Purpose: To make use of an existing rat model for PTSD where individual differences in long-term behavioral responses to the scent of a predator results in extreme responses for 25% of animals thought to be analogous to "PTSD," while 25% of the animals show stress resistance. That the extreme behavioral effects can be blocked by cortisol administration prior to stress appears similar to observations in trauma survivors of the prophylactic effects of cortisol. The animal model has not been used to identify CNS gene expression patterns or other biomarkers underpinning biological mechanisms of individual differences in reactivity.

**15. SUBJECT TERMS**

Posttraumatic stress disorder, animal models, gene expression

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| U | U | U | UU | 10 | **19b. TELEPHONE NUMBER** *(include area code)* |

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**Annual Report for Army Award W81XWH-08-2-0021, entitled "Molecular Mechanisms Underlying Individual Differences in Response to Stress in a Previously Validated Animal Model of PTSD"**

*Revised and Resubmitted 1.19.10*

## INTRODUCTION:

More than 1.5 million troops have been deployed to Iraq, and approximately 30% of returning Warfighters have post-traumatic stress disorder (PTSD). PTSD is a disorder that results from exposure to stressful events and results in significant and often long-term disability. It is at present unclear why some individuals exposed to stress develop PTSD while others exposed to similar challenges do not. One possibility is that pre-existing biological differences among individuals may predispose some and not others to develop PTSD. The goal of the present study is to identify potential biological differences that may predispose to PTSD using a validated PTSD rodent model: where individual differences in long-term behavioral responses to the scent of a predator (cat urine) results in long-term extreme behavioral responses for 25% of the animals thought to be analogous to PTSD and 25% of the animals show stress resistance (Cohen et al., 2004). Our overall objective is to identify genes showing altered expression in brain areas implicated in PTSD (anterior cortex, amygdala, and hippocampus) that relate specifically to individual differences in fear reactivity (Shin, Rauch & Pitman, 2006). We are also attempting to relate these brain changes in gene expression to those present in simultaneously obtained blood samples. Our final aim is to determine whether identified target genes are affected by a treatment that reduces stress reactivity - cortisol administration prior to stress exposeure (Moriceau et al., 2004; Schelling et al., 2006). We hypothesize that there will be different RNA expression profiles in the brains of rats showing extreme vs. minimal long-term behavioral responses to stress. We also expect that some genes differentially expressed in the brain will also differ in blood samples, but brain and blood may express unique markers associated with the behavioral phenotypes. We further hypothesize that some differences will no longer be present following cortisol pretreatment. These studies offer the opportunity to improve veteran's health by increasing our understanding of the biological basis of PTSD and may also suggest improved methods of treatment or prevention.

## BODY:

During the past year animal subcommittee approval was obtained and Dr. Hagit Cohen's research team at Ben Gurion University of the Negev performed the behavioral work for the grant. The behavioral results for Experiment 1 confirmed that the behavioral stress paradigm produced three distinct behavioral phenotypes following a single exposure to predator odor in both male and female rats. The behavioral responses of 28 adult male and 28 adult female Sprague-Dawley rats (an outbread strain) were examined. The procedures and outcomes of the behavioral work for Experiment 1 for both male and female rats are summarized below.

Male Rats

Male rats were randomly assigned to two groups: Naive control rats (n-5) were exposed to fresh, unused cat litter for 10 min while rats in the stress exposed condition (n=23) were exposed to a predator scent (cat urine) for 10 min. Behavioral responses were assessed 7 days after predator stress scent (PSS exposure). The behavioral paradigms used were the acoustic startle response (ASR) paradigm and the elevated plus maze (EPM). In the latter paradigm rats are placed on an elevated maze containing both covered and uncovered arms. Anxiety/fear is quantified in this paradigm by assessing the relative time rats spend in the covered and uncovered arms with an increased proportion of time spent in covered arms indicating increased fear/anxiety. The key variables of interest in the acoustic startle paradigm are startle amplitude following exposure to a 110 dB sound burst, and the degree to which the startle response declines over multiple trials (startle habituation). These measures are of interest because PTSD is characterized, in part, by heightened startle responses amongst those who suffer from the disorder.

Cutoff behavioral criteria (CBC) were used to divide the PSS exposed rats into three behavioral phenotypes: extreme behavioral responders (EBR), partial behavioral responders (PBR) and minimal behavioral responders (MBR). Two behavioral measures were used to define the cutoff behavioral criteria (CBC's):, 1)) fearful behavior on the EPM and 2) non-habituated exaggerated startle reaction. These were selected to constitute the basis for the behavioral criteria for a number of reasons. First, each has been shown to be a valid measure of stress-responses in numerous studies (Adamec, Shallow & Budgell, 1997; Garrick et al, 1997), and secondly, each is well defined and easily quantified.

To maximize resolution and minimize false positives, extreme responses to both the EPM and for inclusion in the EBR group (anxious, fearful and hyper-vigilant, i.e. PTSD-like symptoms), whereas a negligible degree of response to both was required for inclusion in the MBR group. The validity of the criteria have been previously been verified by ascertaining that the vast majority (<90%) of unexposed control animals conform to the criteria for MBR (unaffected by test procedures) and less than 3%, to the criteria for EBR (Cohen et al., 2006).

The assessment of behavioral phenotypes was performed in two steps. Prior to distinguishing the differently affected sub-groups, we performed a preliminary data assessment to verify overall response of the exposed population, ascertaining that exposure to the stressor did, in fact, have a significant overall behavioral effects on the exposed rats as a group, compared to controls and elicited a range of individual behavioral changes. Behavioral changes such as extremely compromised exploratory behavior on the plus-maze and markedly increased startle reaction that does not habituate were taken to reflect anxiety-like behaviors, i.e. fearfulness and hyper vigilance.

In keeping with the work of Blanchard and Blanchard (Blanchard et al., 1990; Blanchard et al., 1998; Blanchard et al., 1993), Adamec (Adamec, 1997; Ademec et al., 1998; Adamec, Blundelll & Burton 2003; Adamec et al, 1999; Adamec & Shallow, 1993) and Dr. Cohen's previous studies (Cohen et al., 1996; Cohen, Kaplan & Kotler, 1999; Cohen & Zohar, 2004; Cohen, Zohar and Matar, 2003; Cohen et al., 2005; Cohen et al. 2004), the proportion of individuals displaying behaviors fulfilling criteria for EBR at 7 days were considered to demonstrate long-term and persistent changes. Beyond this time frame no significant changes in the prevalence of EBR has been observed.

Having established that the stressor had an effect on the rats and that not all animals responded to it in the same manner, we went on to focus on individuals that demonstrated extremes of behavioral change (EBR), or virtually none (MBR). The cut-off criteria on each behavioral paradigm were as follows:

EBR:

1. Five minutes (entire session) spent in closed arms and no entries into the open-arms on the EPM.
2. Mean startle amplitude > 700 units and no habituation over time.

MBR:

1. One minute or less time spent in closed arms and eight or more open-arm entries on the
2. Mean startle amplitude < 700 units and habituation of the response over trials.

PBR:

1. Behavioral responses intermediate to those above.

Within the PSS-exposed population a broad range of variation in behavioral response was observed and EBR, PBR subgroups were identifiable (see Fig. 1). Rats were subdivided into three groups based on the reflecting magnitude of response according to the CBC's, focusing selectively on EBR, PBR and MBR.

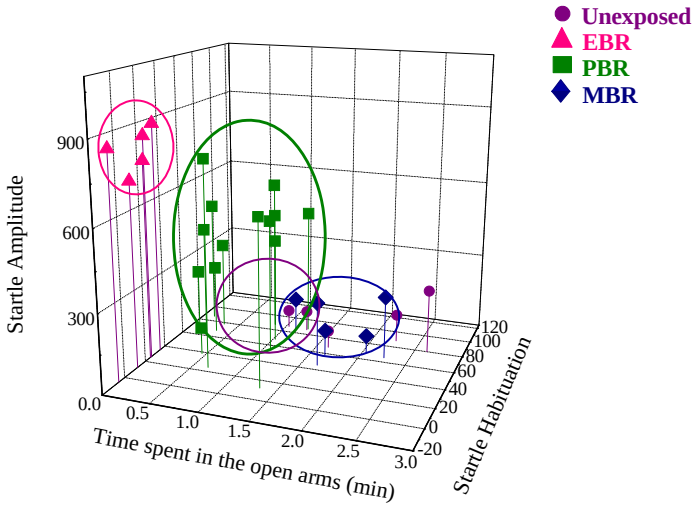


**Figure 1.** *Male rat elevated plus maze (EPM) behavior and the acoustic startle responses of extreme behavioral responders (EBR), partial behavioral responders (PBR), minimal behavioral responders (MBR) and unexposed control animals one week after a 10 min exposure to predator stress scent (cat urine). The X-axis represents time spent in the open arms of the EPM, the Y-axis represents acoustic startle amplitude, and the Z axis represents startle habituation.*

The graphic representation of the data from both paradigms (EPM and ASR) reveals two obvious and rather distinct features. Firstly, it is clear that PSS exposure alters the response of the majority of individuals to at least some degree. A single ten-minute exposure to PSS significantly increased anxiety-like avoidance of open spaces as compared to unexposed controls. Values for time spent in the open arms ($F_{(1,26)} = 8.4$, $p < .0075$) were significantly decreased after stress exposure, as compared to control conditions. There were no differences in total exploration of the maze between groups. This result suggests overall anxiety and avoidance of exploration in the open arms, as opposed to an impairment of

locomotion/exploration. Stress exposure also significantly increased the mean startle amplitude and caused a significant deficit in the habituation of ASR in exposed rats as compared to controls ($F_{(1,26)}$ = 4.8, $p < .004$ and $F_{(1,26)} = 8.2$, $p < .0085$ respectively). Secondly the cluster of individuals that forms in the upper left hand corner of the graph (i.e. more extreme responses to exposure) is quite distinct from the majority of individuals.

The pooled behavioral data for the entire PSS-exposed populations were re-examined according to the CBCs. In the exposed group 5 rats (21.8%) fulfilled criteria for EBR, whereas 5 rats (21.8%) were characterized as MBR. All other rats fell between the CBC's for the extreme groups, and were defined as PBR animals. In the control group no rats (0%) fulfilled criteria for EBR.

Female Rats

Female rats were randomly assigned to two groups: Naive control rats (n-5) were exposed to fresh, unused cat litter for 10 min while rats in the stress exposed condition (n=24) were exposed to the PSS for 10 min. Behavioral responses were assessed 7 days later and behavioral classification was performed using the methods described above.

Similar to male rats, the time spent by females in the open arms of the EPM ($F_{(1,27)} = 5.2$, $p < .035$) were significantly decreased after stress exposure, as compared to the control condition, and there were no differences in total exploration of the maze between groups. Stress exposure also significantly increased the mean startle amplitude for females and caused a significant deficit in the habituation of ASR in exposed rats as compared to controls ($F_{(1,27)} = 12.1$, $p < .002$ and $F_{(1,27)} = 6.03$, $p < .025$ respectively)). Secondly the cluster of individuals that forms in the upper left hand corner of the graph (i.e. more extreme responses to exposure) is quite distinct from the majority of individuals.

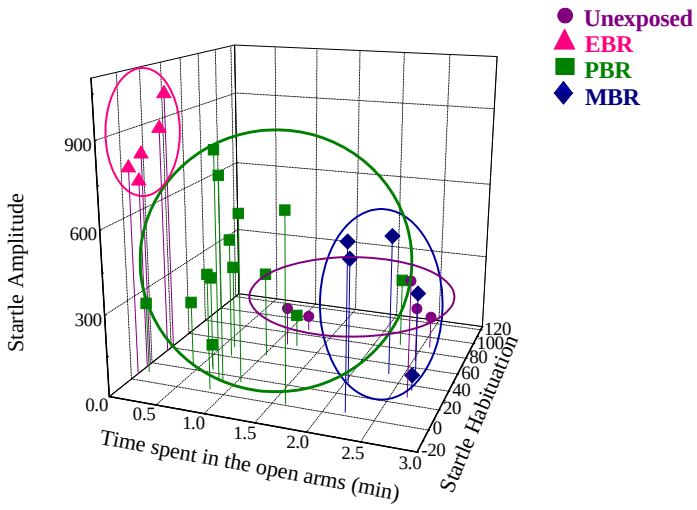


**Figure 2.** *Female rat elevated plus maze (EPM) behavior and the acoustic startle responses of extreme behavioral responders (EBR), partial behavioral responders (PBR), minimal behavioral responders (MBR) and unexposed control animals one week after a 10 min exposure to predator stress scent (cat urine). The X-axis represents time spent in the open arms of the EPM, the Y-axis represents acoustic startle amplitude, and the Z axis represents startle habituation.*

The pooled behavioral data for entire PSS-exposed populations were re-examined according to the CBCs (Fig. 2). In the exposed group 5 rats (20.8%) fulfilled criteria for EBR, whereas 5 rats (20.8%) were characterized as MBR. All other rats fell between the CBC's for the extreme groups, and were defined as PBR animals. In the

control group no rats (0%) fulfilled criteria for EBR.

Following the completion of the behavioral work described above, animals were sacrificed, trunk blood samples were collected and brain micro-dissection was performed to isolate the anterior cortex, amygdala, and hippocampus. Samples were then shipped to Dr. Buxbaum's laboratory at Mount Sinai School of Medicine.

A total of 90 brain samples from the 30 rats classified as EBR, MBR or control animals have undergone genome-wide gene expression profiling to identify genes that are differentially expressed. Each group consisted of 5 male and 5 female rats. We are currently analyzing the gene expression data. We have not yet run the analysis of trunk blood to accomplish the aim of comparing gene expression in blood to that of the brain regions of interest.

Rather than immediately progressing to our second proposed experiment in which we ere to examine the effects cortisol pretreatment on behavior and gene expression following PSS exposure, we elected to do a validation study as we have been advised that this is the most scientifically accurate and cost effective way to accomplish our aims. The behavioral work for the validation study has been performed yielding a second EBR group consisting of 12 female, and 13 male rats, an MBR group consisting of 12 female and 10 male rats and a control group consisting of 10 female and 10 male rats. Pending results of the initial genome-wide microarray, and the validation study, we will redo the study with a cortisol pretreatment condition, focusing on the identified 'target' genes. This will likely not happen during the funding period.

**KEY RESEARCH ACCOMPLISHMENTS:**

- Animal subcommittee approval for the study was sought and obtained.
- Use of the fear paradigm has been established and the usefulness of this paradigm for our studies has been established.
- The behavioral work for Experiment 1 has been completed.
- Biological samples from Experiment 1 have been obtained and brain dissection has been performed.
- RNA extraction and gene profiling analysis has been performed on the 90 brain samples obtained from the 30 EBR, MBR and control animals from Experiment 1.
- Behavioral data have been collected for a second validation study intended to bolster the results obtained from the originally proposed first experiment.

**REPORTABLE OUTCOMES:**

There are no reportable outcomes to date, as the gene expression data have not been analyzed. We will be paying for the samples in the next 2 weeks, at which time we will be able to begin data analysis.

**CONCLUSION:**

The usefulness of the behavioral fear paradigm has been established, behavioral analysis has been completed and gene expression analyses on the biological specimens collected is ongoing. We have not yet completed analysis of the biological data. When we finish running

the analyses for Experiment 1 and the subsequent validation study, we will have accomplished our objective of identifying genes that are differentially expressed in blood and three brain regions associated with fear responses in rats that develop PTSD-like symptoms, and those that are resistant to developing these symptoms. After the completion of the replication study (which was not budgeted for, but will be necessary to confirm our findings) we will repeat the study using a cortisol pretreatment. It is hoped that the results obtained from this work will increase our understanding to the biology of PTSD and facilitate the detection of PTSD susceptibility and the treatment of the disorder.

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**Appendices**
None